IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: 1633	**Customer No.: 035811**
Examiner	: Maria Marvich	
Serial No.	: 10/764,628	
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Inventors	: Véronique Trochon-Joseph	Docket No.: 1002-04
	: He Lu	
	: Claudine Soria	Confirmation No.: 9953
Title	: METHOD OF INHIBITING	
	: ANGIOGENESIS OR	
	: INVASION OR FORMATION	
	: OF METASTASES	

DECLARATION OF LLUIS MIR UNDER RULE 132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir :

I, Lluis Mir, residing at 22 allée des Vaupépins 91370 Verrières le Buisson (France), declare and say that:

I am citizen of France.

I have a PhD degree and graduated from the Ecole Normale de Paris in France in 1978.

I am Director of the Vectorology and Anti-cancerous Therapeutics Research Unit of the Integrated Research Cancer Institute in Villejuif (France).

I am the author of the article "Nucleic Acids Electrotransfer-Based Gene Therapy (Electrogenetherapy): Past, Current, and Future" published in Mol Biotechnol (2009) 43:167–176.

I am aware that the Patent Office quotes page 170, last paragraph, and page 171, paragraph 2, of the article considered "local transfer is required as concentration of DNA is low and this is sensitive to dilution, and thus injection and electric pulses must be limited to the target site".

However, the descriptions made in pages 170 and 171 of the article concern "a method of gene transfer" and not at all "the efficacy of a therapeutic approach based on gene electrotransfer".

Indeed, one can see that the two following statements mentioned in the rejection:

- "First of all, it is necessary to recall that DNA electrotransfer, like all the other physical methods for gene transfer, is not a systemic method of gene transfer, but, a strictly local one"; and
- "The second reason is less stringent: it is advised to inject locally the DNA because, due to the large size of the DNA molecule, its concentration is always very low and thus very sensitive to dilution"

strictly concern the "gene transfer method".

In this article, I made no description of gene electrotransfer applications. I just quoted two previous reviews by myself and co-workers where the reader can find the main applications of the technology described in the article in Mol Biotechnol (2009) 43:167–176.

Indeed, the applications of gene electrotransfer were already described in anterior documents. For example, on the top of page 172 of the Mol Biotechnol article, one can find:

"As a matter of fact, gene transfer by electric means has been tested and successfully achieved in a large number of tissues in many animal species, including the usual laboratory species (mice, rats, rabbits), cattle, pets, and exotic animal species, as extensively reviewed, for example, by Andre and Mir [57], and Mir et al. [58]".

In these reviews:

57. Andre, F., & Mir, L. M. (2004). DNA electrotransfer: its principles and an updated review of its therapeutic applications. Gene Therapy, 11(Suppl 1), S33–S42; and

58. Mir, L. M., Moller, P. H., Andre, F., & Gehl, J. (2005). Electric pulse-mediated gene delivery to various animal tissues. Advances in Genetics, 54, 83–114;

the reader will easily find systemic effects of the locally delivered plasmids.

For example, reference 57, page S36, section "Therapeutic applications already developed in preclinical trials", paragraphs 2 and 3, states:

"The main general application is immunotherapy (48%; 54/113). Cancer treatment (38%; 43/113), metabolic disorders or metabolism modification (17%; 19/113) and correction of organ or site-specific diseases (14%; 16/113) are the three other frequent applications. Monogenetic diseases (9%; 10/113), cardiovascular diseases (9%; 10/113) and analgesia (2%; 2/113) are other applications also found in the literature. It must be noted that each of these applications includes the use of a large variety of genes. In this respect, it is necessary to point out that the genes of proteins involved in the immune system responses have been the most usually transferred genes for vaccination, cancer, treatment of arthritis, immunological protocols, etc. These genes include those coding for the IL-2, IL-4, IL-10, IL-12, IL-18, IL-18 binding protein, soluble TNF receptor, GM-CSF, tumour epitopes, the HIV gag gene, recombinant monoclonal antibodies, mycobacterial antigens, etc. The details are listed in the Table 2. In fact, this observation must be related to the fact that in many cases the transfected tissue is the skeletal muscle, used as a cell factory for the production of factors that will act systemically on distant targets."

In particular, Tables 1 and 2 of reference 57 show the vast number of applications in which the transfected tissue is the muscle and the effect is either systemic or distant. For instance, cardiothrophin gene has been electrotransfered in muscle for achieving treatment of neuron degeneration; HGF has been electrotransfered in muscle for achieving kidney or liver regeneration.

Accordingly, my articles show that antitumor and antimetastatic effects can be anticipated from the intramuscular injection of the gene coding for the AMEP protein.

The undersigned Declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed on May 3rd, 2012

Lluis MIR